

Immunofluorescence staining for the detection of *Neisseria gonorrhoeae* in women

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Although the use of a fluorescent antibody (FA) staining technique to demonstrate *N. gonorrhoeae* in secretions from patients with gonorrhoea was first described as long ago as 1959 by Deacon, Peacock, Freeman, and Harris, it does not seem to have gained wide acceptance in routine diagnosis. In our laboratory direct and delayed FA methods were first assessed in 1963 (Fry and Wilkinson, 1964). In women the direct method gave more positive results than the delayed which gave slightly more positives than Gram-stained smears or cultures on a non-selective medium; it was possible to demonstrate gonococci by the delayed FA method when the organism was overgrown by *Proteus* on conventional culture plates.

Lucas, Price, Thayer, and Schroeter (1967) obtained a similar number of positives in females with the delayed FA method to that with cultures on Thayer-Martin medium. Lind (1969) also obtained a close correlation in both males and females between the delayed FA test and culture on a selective medium, although in a previous study of specimens from females (Lind, 1967) the delayed FA method was superior to culture on a non-selective medium; in the latter study the direct FA method did not offer any advantage over Gram-stained smears. Thin, Williams, and Nicol (1971) found that, in the examination of cervical and urethral specimens from women, direct as well as delayed FA tests gave more positive results than Gram-stained smears or cultures; however, they point out that selective media were not used, and when the plates had only a few colonies after 16 to 24 hrs' incubation all the material may have been removed for the delayed FA test. On the other hand, Martin, Peacock, and Thayer (1965), testing women, found the delayed FA method inferior to cultures on both selective and non-selective media. McGill, Moffett, Masterton, and

Schofield (1969) reported far fewer positive results on specimens from women tested by the delayed FA method than by either Gram-stained smears or cultures.

The superiority of the delayed FA technique over conventional culture in the case of specimens from the rectum has been noted by Lind (1967), and by Phillips, Humphrey, Middleton, and Nicol (1972), but Martin and others (1965) found it to be inferior to Thayer-Martin selective medium for rectal specimens as well as those from other sites.

Much of this variation in results is probably related to differences in culture media, in technique, and in the specificity of the antiserum used in the FA tests (Reyn, 1969). In view of these rather conflicting results, it was decided to reassess the value of FA tests in the diagnosis of gonorrhoea in women.

Patients and methods

PATIENTS

Three series of women were tested:

- (1) The direct FA test was compared with conventional tests on cervical specimens from 66 gonorrhoea contacts.
- (2) Direct and delayed FA tests were compared with conventional tests on cervical and rectal specimens from 99 gonorrhoea contacts.
- (3) Direct and delayed FA tests were compared with conventional tests on cervical and rectal specimens from 112 consecutive new patients not known to be gonorrhoea contacts.

CULTURAL METHODS

Specimens were taken from the urethra, cervix, and rectum on charcoal-treated cotton swabs, and transported in Stuart's medium. On receipt in the laboratory they were plated on Oxoid brain heart infusion agar (CM 375) with 10 per cent. horse blood containing vancomycin 3.0 µg./ml. and colisti-methate sodium 7.5 µg./ml. Plates were incubated at 36°C. for 48 hrs in an atmosphere of 5 per cent. CO₂ and gonococci identified by colonial

morphology, examination of Gram-stained smears, the oxidase test, and fermentation tests. Gram-stained smears of secretions were examined in the clinic at the time the patients were examined. The material for the conventional tests was taken before that for the FA tests.

FLUORESCENT ANTIBODY TESTS

An antigonococcal serum was prepared in three rabbits against mixed suspensions of thirty freshly isolated strains of gonococci. A suspension containing approximately 3.6×10^9 organisms per ml. was emulsified in an equal volume of Freund's complete adjuvant and injected subcutaneously into each thigh. One month later 0.15 ml. and 0.2 ml. of the gonococcal suspension was injected intradermally at weekly intervals. This produced a marked local reaction and the animals were bled out. The complement-fixation titre of the pooled serum was 1 in 1,280.

CONJUGATION

Globulins were precipitated by half saturation with ammonium sulphate and after dialysis applied to a DEAE column and eluted with 0.02M phosphate buffer, pH 8.1. Fractions of the fall-through peak were pooled and concentrated to the original volume of serum. Immunoelectrophoresis showed a single line with the mobility of a gamma globulin. This was conjugated with FITC and unreacted dye removed by passage through a Sephadex G25 column. The F/P ratio of the conjugate was 9.8 µg. FITC/mg. protein. It was stored in aliquots at -20°C . Tests showed that a 1 in 8 dilution in normal rabbit serum gave bright staining of gonococci in direct smears of secretions and suspensions from cultures of gonococci but did not stain other commensal *Neisseria* or staphylococci.

DIRECT FA TESTS

Thin smears of secretions were spread within inscribed circles 1 cm. in diameter on scrupulously cleaned slides, allowed to dry in the air, and sent to the laboratory. In the direct FA test, only morphologically typical intracellular organisms showing definite fluorescence were recorded as positive. If only extracellular fluorescing organisms were seen, the result was recorded as doubtful.

DELAYED FA TESTS

Material was inoculated directly on to slopes of the isolation medium (which was the same as for conventional culture) in bijou bottles and incubated overnight at 36°C . Thin films of the mixed growth were made and stained. If negative, the culture was incubated for a further 24 hrs and examined again.

Slides were fixed for 3 min. in 3 per cent. formalin in buffered saline pH 7.2 (PBS), washed in two changes of distilled water for 5 min., and allowed to dry. A drop of a 1 in 8 dilution of the conjugate in inactivated normal rabbit serum was spread over the circular area and left for 30 min. at 35°C . in a moist chamber. The excess was washed off and the slides soaked in two changes of PBS

for 5 min. each, rinsed in distilled water, and mounted in 80 per cent. glycerol in PBS. No counterstain was used as this conjugate gave only minimal staining of leucocytes.

Results

SERIES 1

Gonorrhoea contacts. Direct FA tests on cervical secretion alone (Table I)

Twenty of the patients were classed as primary (source) contacts, and 46 as secondary contacts or as having insufficient information to assess their contact category. Cervical tests were positive or doubtful by one or more methods in 39 (59 per cent.) of the whole group. Sixteen (80 per cent.) of these were among the primary contacts, and 23 (50 per cent.) among the others. The direct FA test appeared rather more sensitive than the examination of Gram-stained smears. In one patient the direct FA test was the only one positive, and in three the only finding was a doubtful FA result. One patient with a positive culture and three with doubtful Gram smear results had negative FA tests.

TABLE I *Comparison of direct FA tests with Gram-stained smears and cultures from the cervix in 66 female contacts*

Type of contact	Result	Gram smear	Culture	Direct FA
Primary (20)	Positive	9	11	11
	Doubtful	4	—	2
	Negative	7	9	7
Secondary or Undetermined (46)	Positive	13	17	19
	Doubtful	3	—	4
	Negative	30	29	23

SERIES 2

Gonorrhoea contacts. Direct and delayed FA tests on cervical and rectal secretions (Table II)

These included 39 primary and sixty secondary or undetermined contacts. Tests were positive or doubtful by one or more methods in 72 (73 per cent.) of the whole group; 33 (85 per cent.) were among the primary contacts and 39 (65 per cent.) among the others. Direct FA tests on cervical and rectal secretions gave rather more positive or doubtful results (73) than the Gram-stained smears (66). Delayed FA tests gave slightly more positive results than conventional cultures of cervical secretions, but the difference was much more marked in the case of rectal cultures in which the delayed FA test was positive in forty, compared with only 25 on conventional cultures. Although trimethoprim was not incorporated in the latter, overgrowth with *Proteus*

TABLE II *Comparison of direct and delayed FA tests with Gram-stained smears and cultures from the cervix and rectum in 99 female contacts*

Type of contact	Result	Cervix				Rectum			
		Gram smear	Culture	Direct FA	Delayed FA	Gram smear	Culture	Direct FA	Delayed FA
Primary (39)	Positive	18	24	25	29	7	12	14	20
	Doubtful	—	—	1	—	2	—	—	—
	Negative	21	15	13	10	30	27	25	19
Secondary or Undetermined (60)	Positive	29	30	24	29	7	13	6	20
	Doubtful	1	—	2	—	2	—	1	—
	Negative	30	30	34	31	51	47	53	40

was not a problem and did not account for the difference. In four of the contacts the FA tests were the only ones positive; in two of these patients the direct FA tests were positive and in all four the delayed FA test gave a positive result (with specimens from the rectum in two, from the cervix in one, and from both sites in one). Two patients had positive conventional but negative FA tests.

SERIES 3

Direct and delayed FA tests on cervical and rectal secretions from 112 consecutive new patients not known to be gonorrhoea contacts (Table III)

Tests were positive or doubtful in nine (7.5 per cent.). Again, the delayed FA tests on cervical and rectal secretions gave more positive results than the other methods. In four patients the FA results were confirmed by the isolation and identification of gonococci from either the cervix or rectum. In five patients one or more of the FA tests were positive or doubtful, but the conventional cultures and Gram-stained smears were negative. Three of the male contacts had been treated for non-specific urethritis, but there was no information on the other two. In three of the women the delayed FA test was positive on both cervical and rectal specimens and in one of these the direct FA test on the cervix was reported as doubtful. Since fluorescing organisms were found in both sites tested, it is thought probable that they were, in fact, gonococci; one patient defaulted and the other two were treated with oxytetracycline

because their contacts had non-specific urethritis. In one patient only the delayed FA test on the rectal specimen was positive, but this was negative when repeated. In another the direct FA test on the rectum was doubtful and the organisms were noted to be atypical in morphology; she was treated with oxytetracycline because her contact had non-specific urethritis. If the tests in the last two, or in all of these five patients, are regarded as giving false positive results because of the lack of confirmation by conventional methods, the incidence of such results is between 1.8 and 4.5 per cent. in this group of patients who were not suspected of having gonorrhoea.

Discussion

In this study the delayed FA method was found to be more sensitive than the direct FA technique. This difference was most marked in the case of rectal specimens. The direct method was rather more sensitive than the examination of Gram-stained smears. The whole area of the smear within the circular area was scanned before a negative result was recorded; this took about 30 minutes to carry out, which is considerably longer than could be devoted to examining the comparable Gram-stained smears. This makes the direct method impracticable as a routine procedure. The delayed method is simple and quick to perform, but it was noted that a good many specimens were negative when examined after incubation overnight but gave unequivocal positive

TABLE III *Comparison of direct and delayed FA tests with Gram-stained smears and cultures from the cervix and rectum of 112 unselected patients not known to be gonorrhoea contacts*

Result	Cervix				Rectum			
	Gram smear	Culture	Direct FA	Delayed FA	Gram smear	Culture	Direct FA	Delayed FA
Positive	4	3	5	7	2	2	3	7
Doubtful	—	—	1	—	—	—	1	—
Negative	108	109	106	105	110	110	108	105

results if the cultures were re-incubated for a further 24 hrs. This was more noticeable with rectal than with cervical cultures, sixteen of 47 rectal cultures being positive only after re-incubation, compared with eight of 65 cervical cultures. This probably reflects a smaller initial inoculum of gonococci from the rectum than from cervical material. The additional yield of positive results warrants the routine re-incubation of specimens found negative at the first examination after incubation for 18 to 24 hrs.

Although we found that in the case of rectal specimens the delayed FA test gave many more positive results than conventional culture, Ødegaard (1972) has isolated gonococci from this site after transport of specimens in solid Stuart's medium and conventional culture on a selective medium (containing vancomycin, colistin, nystatin, and trimethoprim) in 62 per cent. of 222 women with gonorrhoea.

Overall, the increase in yield of cases of gonorrhoea obtained by employing the delayed FA method in known gonorrhoea contacts was about 5 per cent. It is difficult to assess the value of the delayed FA test in women not known to be gonorrhoea contacts. In our series this test seemed to detect more cases than the conventional methods, but when only one site is positive by the delayed FA method the possibility of a false positive result must be considered.

Summary

- (1) Fluorescent antibody tests for gonococci were carried out on two series of 66 and 99 female contacts of males with gonorrhoea, and on 112 unselected female patients who were not known to be contacts. The results were compared with those of Gram-stained smears and conventional cultures.
- (2) The delayed FA method was found to be more sensitive than the direct technique, although the latter was more sensitive than the examination of Gram-stained smears. The delayed technique gave many more positive results than conventional cultures of rectal secretions.
- (3) Delayed FA cultures should be incubated for 48 hrs before being discarded as negative.
- (4) In five of the 112 unselected patients (4.5 per cent.), positive or doubtful results in the FA tests were found which were not confirmed by conventional cultures or Gram-stained smears. This was

the case in 8 (5 per cent.) of the 165 patients who were known to be contacts of patients with gonorrhoea.

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Détection de *Neisseria gonorrhoeae* chez la femme par le marquage immuno-fluorescent

SOMMAIRE

- (1) Des tests à l'anticorps fluorescent gonococcique furent effectués sur deux séries de 66 et 99 femmes ayant été en contact avec des hommes atteints de gonococcie et chez 112 femmes non choisies, non connues pour avoir eu de tels contacts. Les résultats furent comparés avec ceux de la coloration des sécrétions au Gram et avec les cultures conventionnelles.
- (2) La méthode retardée (=indirecte, N.d.T.) fut trouvée plus sensible que la méthode directe, quoique cette dernière fut plus sensible que l'examen des sécrétions colorées au Gram. La technique retardée donna bien plus de résultats positifs que la culture conventionnelle en ce qui concerne les sécrétions rectales.
- (3) Par la méthode retardée, les cultures doivent être incubées pendant 48 heures avant d'être déclarées négatives.
- (4) Chez cinq des 112 malades non choisies (4,5 pour cent), les tests de fluorescence furent positifs ou douteux, qui ne furent pas confirmés par les cultures conventionnelles ou l'examen de lames colorées au Gram. Ceci fut aussi observé chez 8 (5 pour cent) des 165 malades qui étaient connues pour avoir été en contact avec des gonococciques.